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ORIGINAL ARTICLE

Optimization of ultrasound assisted hypochlorite oxidation of corn starch by response surface methodology

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Abstract

The study on the influence of active chlorine content (0.25 g/25 g starch–0.75 g/25 g starch), amplitude of ultrasound (50% to 100%), sonication time (10 min to 30 min) and their interactive effects on the degree of oxidation of hypochlorite-oxidized corn starch was conducted and also the regression models to predict the degree of starch oxidation, carbonyl and carboxyl contents were developed by employing a three-level three factorial Box-Behnken design. It was found that active chlorine content showed the greatest influence on degree of starch oxidation, followed by sonication time and amplitude of ultrasound. Verification of the proposed regression model was performed and the predicted values obtained from the model for both responses for samples prepared with different oxidation conditions showed a very good agreement with the measured values. This indicates that models proposed are capable of predicting degree of starch oxidation.

Keywords: Starch oxidation; Box-Behnken design; sonication; carbonyl content; carboxyl content

Introduction

Starch is the main storage polysaccharide in plants. It is commonly used in food and non-food applications due to the fact that it is a cheap and versatile biopolymer because its physicochemical properties can be manipulated through genetic, enzymatic, chemical, and physical modifications (Jobling, 2004). These modifications would help to enhance starch positive attributes and/or to minimize its defects (Kaur *et al.*, 2012).

One of the common modification methods of starch is oxidation. Oxidized starch is well known for its unique functional properties such as low viscosity, high stability, and excellent clarity as well as enhanced film forming and binding properties (Kuakpetoon and Wang, 2006). Reagents such as permanganate (Takizawa *et al.*, 2004), hydrogen peroxide, hypochlorite (Sangseethong *et al.*, 2010) and oxygen (Ye *et al.*, 2011), have been used to oxidize starch, however, sodium hypochlorite is the oldest and most popular commercial oxidizing agent used (Kuakpetoon and Wang, 2001; Sandhu *et al.*, 2008). According to Wurzburg (1986), there are two main reactions take place during starch oxidation. Firstly, starch hydroxyl groups are oxidized to carbonyl groups and then to carboxyl groups and these occur mainly on the hydroxyl groups at the C-2, C-3 and C-6 positions. Secondly, oxidation causes depolymerization of starch molecules by primarily hydrolyzing amylose and amylopectin at α-D-(1→4) glycosidic linkages. The extent of hypochlorite oxidation is affected by pH, temperature, hypochlorite concentration, starch molecular structure and starch source.

The use of ultrasound in the food industry has been extensively studied for the past decades (Mason *et al.*, 1996; Chang and Chen, 2002). Nowadays, it has been widely used in food processing, food preservation and extraction field (Chemat *et al.*, 2011). When high intensity ultrasound is transmitting through a liquid medium, the dissolved gas will serve as nuclei to form cavities. Under the influence of an oscillating pressure field caused by the ultrasound during sonication, these microbubbles will grow to a critical size and collapse violently, generating extremely high localized temperature and pressure at the vicinity of the collapsing bubbles (Leighton, 1994). The implosive collapse of a bubble will induce high pressure gradients and causing the surrounding liquid layers rushes in at

high local velocities. Solvent and solute molecules present within the bubbles may dissociate to form radicals that in consequence will initiate chemical reaction in the system (Czechowska-Biskup *et al.*, 2005).

From the literature, it was found that Wang and Wang (2003) had studied the physicochemical properties of common and waxy corn starches oxidized by different levels of sodium hypochlorite. They reported that the degree of starch oxidation was positively correlated to the levels of oxidant used. Jambrak *et al.* (2010) had examined the effect of various ultrasound treatments such as ultrasound devices, intensity and treatment time on rheological and some physical properties of corn starch. They revealed that starches prepared at different combination of ultrasound treatments exhibited different properties. A study by Zuo *et al.* (2009) showed that the pasting behaviour of starch suspensions would be affected by sonication conditions such as sonication time and temperature.

Response Surface Methodology (RSM) is an empirical model method applied to evaluate the relationship of a set of controlled experimental variables and observed responses. One of the advantages of RSM is that plenty of information can be gained from a small number of experiment runs. Details or information about the interactive effect of the independent variables on the response and about the process or reaction can be obtained from the empirical model that relates the response to the independent variable. One of the popular experimental design is Box-Behnken design. Box-Behnken designs are available for 3 to 7 factors and it requires only 3 levels, code as -1, 0 and +1. It allows prediction of the combined effects of three controlled parameters. Fewer numbers of runs is required as it minimizes the number of factor combination (Lahlali *et al*., 2008; Khajeh, 2009; Wu *et al*., 2012). Studies or works regarding the employment of RSM in starch oxidation to relate the degree of starch oxidation with oxidant levels, sonication time and ultrasound amplitude are rarely found. Hence, the aim of the present work was to evaluate and model the influence of active chlorine content, amplitude of ultrasound and sonication time on the degree of oxidation of hypochlorite-oxidized corn starch. A three-level three factorial Box-Behnken design was employed to examine the effects of these independent variables and their combined effects on the responses, namely carbonyl and carboxyl contents of the modified corn starch. An empirical model correlating the degree of oxidation to the three variables was developed.

Materials and methods

Materials: Commercial corn starch containing 26.47% amylose was purchased from Sim Company Sdn. Bhd, Penang, Malaysia. Sodium hypochlorite (NaOCl) containing 10% active chlorine was used in this experiment. All other chemicals used were of analytical grade.

Sample preparation: Hypochlorite-oxidized corn starches were prepared according to the modified procedure of Chong *et al.* (2013). A 10% corn starch slurry (w/w) was prepared by dispersing 25 g of starch powder in 225 g of deionized water. The pH of starch slurry was adjusted to 9-10 with 2 mol equivalent/L NaOH with consistent stirring using a magnetic stirrer. Three levels of oxidation (0.25 g active chlorine/25 g starch, 0.50 g active chlorine/25 g starch and 0.75 g active chlorine/25 g starch) were obtained by slowly adding NaOCl into the starch slurry within 30 min while maintaining the pH at 9-10 with 0.5 mol equivalent/L H_2SO_4. After the addition of NaOCl, the starch slurry were treated with a 24 kHz probe with 7 mm diameter titanium tip (UP 200h, "Dr. Hielscher" GmbH, Teltow Germany) for 10, 20 and 30 min with power ultrasound of 300W of nominal power with 50, 75 and 100% amplitude, respectively. Then, the starch slurry was adjusted to pH 7.0 with 0.5 mol equivalent/L H_2SO_4; vacuum filtered, washed with deionized water, washed with absolute ethanol and dried in a vacuum oven at 30 °C overnights for up to 48 h.

Determination of carbonyl content: The carbonyl content of native and modified corn starch samples was determined according to the titrimetric method of Kuakpetoon and Wang (2006). An amount of 4 g starch (dry basis) was suspended in 100 mL of distilled water in a 250 mL flask. The starch suspension was gelatinized in a boiling water bath for 20 min and cooled to 40 °C. Then, the pH of the suspension was adjusted to 3.2 with 0.1 mol equivalent/L hydrochloric acid (HCl) and added with 15 mL of hydroxylamine reagent. The flask was capped and placed in a 40 °C water bath for 4 h with slow shaking. The reaction mixture was rapidly titrated to pH 3.2 with standardized 0.1 mol equivalent/L HCl in order to determine the excess hydroxylamine. A blank determination with only hydroxylamine reagent was carried out with the same procedure. The hydroxylamine reagent was prepared by dissolving 25 g of hydroxylamine hydrochloride in 100 mL of 0.5 mol equivalent/L NaOH before adjusting the final

volume to 500 mL with distilled water. The carbonyl content of the starch sample was calculated as follows:

Percentage of carbonyl content = [(Blank-Sample) mL x Acid molarity x 0.028 x 100]/Sample weight (db) in g (1)

Determination of carboxyl content: The carboxyl content of unmodified and modified corn starches was determined following the procedure of Kuakpetoon and Wang (2006). An amount of 2 g starch (dry basis) was mixed with 25 mL of 0.1 mol equivalent/L HCl in a 100 mL beaker and the slurry was stirred for 30 min with a magnetic stirrer at room temperature. The slurry was then vacuum filtered through a filter funnel and the starch sample was washed with 400 mL of deionized water to remove chloride ions from the starch cake. With the aid of deionized water, the starch cake was transferred into a 500 mL beaker, and the volume was adjusted to approximately 300 mL with deionized water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to ensure complete gelatinization. Then, the hot starch solution was adjusted to approximately 450 mL with boiling deionized water and immediately titrated with standardized 0.01 mol equivalent/L NaOH to pH 8.3. The amount of NaOH used in titration was recorded. Blank tests were performed for unmodified starch to correct for inherent acidic substances by stirring 2 g of unmodified starch with 25 mL of deionized water instead of 25 mL of 0.1 mol equivalent/L HCl. The carboxyl content of starch sample was calculated as follows:

Percentage of carboxyl content =[(Sample-Blank)mL x Alkaline molarity x 0.045 x 100]/Sample weight (db) in g (2)

Box-Behnken experimental design: In the present study, a three-level three-factorial Box Behnken experimental design was employed as it required fewer runs than the other designs among all the RSM designs. The experimental levels and codes of chosen variables in this study are presented in Table 1. The low, center and high levels of each variable were designated as -1, 0, and +1, respectively. The run order of the experiments was completely randomized. The experimental parameters of experiment design with its run order are shown in Table 2. Statistical analysis was done with 'DESIGN EXPERT version 5.0.' (Stat-Ease, Inc., Minneapolis, USA). A total of seventeen experimental runs were carried out. Five times center points were replicated in order to estimate the experimental error. The adequacy of the proposed model was studied by testing for Lack of Fit. Significant lack of fit ($P<0.05$) indicates that the proposed model is inadequate and require refining.

Verification of the proposed regression model: In order to determine the validity of the regression equation proposed, two samples with different oxidation conditions were prepared as listed in Table 7. The measured values of the experiment were compared with the predicted values obtained from the regression equation. The estimation capabilities of the proposed regression equations were evaluated by determining the absolute average deviation (AAD) and coefficient of determination (R^2). AAD and R^2 were calculated as follows:

Absolute average deviation, AAD =

$$\left\{\left[\sum_{i=1}^{p}\left(|y_{i,exp}-y_{i,cal}|/y_{i,exp}\right)\right]/p\right\}\times 100 \quad (3)$$

where $y_{i,exp}$ and $y_{i,cal}$ are the experimental and calculated responses, respectively and p is the number of the experimental run.

Coefficient of determination, R^2, =

$$1-\frac{\sum_{i=1-n}(\text{model prediction}_i-\text{experimental value}_i)^2}{\sum_{i=1-n}(\text{average experimental value}-\text{experimental value}_i)^2} \quad (4)$$

where n is the number of experimental data.

Results and Discussion

Determination of the regression model and statistical evaluation: The experimental values (carbonyl and carboxyl contents of the samples prepared) obtained under different conditions are listed in Table 2. A total of seventeen experimental runs were carried out with a replication of center points for five times which was to estimate the experimental error (Maran *et al.*, 2013). The sequential model fitting for carbonyl and carboxyl contents of the samples prepared are shown in Table 3 and Table 4 respectively. Three tests were carried out to determine the adequacy of model. These included Sequential model Sum of Squares, Lack of Fit Tests and Model Summary Statistics. From Table 3 and Table 4, it was found that quadratic model was the most suitable model for the oxidation process because they had high R^2, adjusted R^2, predicted R^2 and low PRESS for both carbonyl and

Table 1: Experimental design levels of chosen variables

Variables	Levels in Box-Behnken design		
	-1	0	+1
Active chlorine content, X_1 (g/25 g starch)	0.25	0.50	0.75
Amplitude of ultrasound , X_2 (%)	50	75	100
Sonication time , X_3 (min)	10	20	30

carboxyl contents of the samples prepared. The Sequential model Sum of Squares also showed that quadratic model was the highest order polynomial where the additional terms were significant as the PRESS value of cubic model could not be defined in the Model Summary Statistics for both responses. Besides, it can be observed that quadratic model had the highest *p*-value for Lack of Fit Tests. Table 5 and Table 6 show the summary of the analysis of variance (ANOVA) of the both responses (carbonyl and carboxyl contents of oxidized starches). It was found that model was highly significant ($p<0.01$) in both responses.

ANOVA results for carbonyl content in Table 5 show that the carbonyl content was being affected high significantly ($p<0.01$) by the active chlorine content and sonication time but it was not significantly affected by the amplitude of ultrasound (%) at 5% probability. This suggests that an increase in active chlorine content or sonication time will lead to an increase in carbonyl content. Besides, it is noted that the interactive effect between the variables was not statistically significant ($p<0.05$) in affecting the carbonyl content. Apart from that, carbonyl content was found not significantly affected by higher level of active chlorine content but it was significantly affected by higher amplitude of ultrasound (%) and longer sonication time. As shown in Table 5, further increase in the amplitude of ultrasound (%) and sonication time will lead to a decrease in carbonyl content.

On the other hand, ANOVA results for carboxyl content in Table 6 reveal that it was being affected high significantly ($p<0.05$) by active chlorine content, amplitude of ultrasound (%) and sonication time. Results in Table 6 indicate that an increase in active chlorine content, amplitude of ultrasound (%) or sonication time will lead to an increase in carboxyl content. It was observed that the interactive effect of active chlorine content with amplitude of ultrasound (%) and active chlorine with sonication time were statistically significant ($p<0.01$) in affecting the carboxyl content. There could be synergistic effect between the interactions of these variables in producing an effect greater than sum of their individual effect to produce starch with higher carboxyl content. Nevertheless, there was not synergistic effect between amplitude of ultrasound (%) and sonication time. Results in Table 6 also show that carboxyl content was being affected high significantly ($p<0.01$) affected by higher level of active chlorine content, amplitude of ultrasound (%) and sonication time. A futher increase in active chlorine content will increase the carboxyl content. However, a reversed trend was observed for higher level of amplitude of ultrasound (%) and sonication time.

The coefficient of determination, R^2 indicates the part of the response variation that is attributed to variations of the factors and their interactions. It ranges from 0 to 1. Closer of R^2 value to 1 is preferred as this indicates the high predictive power of the model (Lahlali *et al.*, 2008). In the present study, the R^2 value of the quadratic regression model for carbonyl content was 0.993 while the quadratic regression model for carboxyl content had the R^2 value of 0.999. This indicates that only 0.007% and 0.001% of the total variations was not explained by the quadratic regression model for carbonyl and carboxyl contents, respectively. Thus, the fit was good between quadratic model and experimental data. The adjusted R^2 corrects the R^2 according to the size of sample and the number of terms in the model. If there are many terms in the model and the size of the sample is not sufficiently high, the adjusted R^2 may be apparently smaller than the R^2 (Liu *et al*., 2004). Our results show that adjusted R^2 for both responses were closed to the corresponding R^2. The adjusted R^2 were 0.9841 and 0.9984 for carbonyl and carboxyl contents, respectively. 'Adequate precision' estimates the ratio of signal to noise. A value greater than 4 is desirable (Lahlali *et al*., 2008). In Table 5 and Table 6, the adequate precision was found to be 32.455 and 110.532 for carbonyl and carboxyl contents, respectively. This showed an -

Table 2: Box-Behnken experimental design for the three independent variables (actual and code) and their responses

Run order	Active chlorine content, X_1 (g/25 g starch)	Ultrasound amplitude, X_2 (%)	Sonication time, X_3 (min)	Carbonyl content (%)	Carboxyl content (%)
1	0.75 (+1)	75 (0)	30 (+1)	0.1332	0.3745
2	0.75 (+1)	50 (-1)	20 (0)	0.1168	0.2793
3	0.50 (0)	50 (-1)	30 (+1)	0.0709	0.1652
4	0.50 (0)	100 (+1)	30 (+1)	0.0696	0.1857
5	0.25 (-1)	75 (0)	10 (-1)	0.0177	0.0389
6	0.25 (-1)	100 (+1)	20 (0)	0.0251	0.0418
7	0.25 (-1)	75 (0)	30 (+1)	0.0355	0.0420
8	0.25 (-1)	50 (-1)	20 (0)	0.0194	0.0385
9	0.50 (0)	75 (0)	20 (0)	0.0806	0.1641
10	0.50 (0)	75 (0)	20 (0)	0.0804	0.1685
11	0.75 (+1)	75 (0)	10 (-1)	0.1095	0.2165
12	0.50 (0)	100 (+1)	10 (-1)	0.0695	0.1208
13	0.50 (0)	75 (0)	20 (0)	0.0826	0.1690
14	0.50 (0)	75 (0)	20 (0)	0.0827	0.1671
15	0.75 (+1)	100 (+1)	20 (0)	0.1224	0.3214
16	0.50 (0)	50 (-1)	10 (-1)	0.0610	0.0928
17	0.50 (0)	75 (0)	20 (0)	0.0805	0.1706

Table 3: Sequential model fitting for carbonyl content of the samples prepared

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value (Prob > F)
Sequential model sum of squares					
Mean	0.093	1	0.093		
Linear	0.019	3	6.275E-03	128.95	< 0.0001
Quadratic	4.976E-04	6	8.293E-05	4.30	0.0388
Cubic	1.295E-04	3	4.317E-05	30.95	0.0032
Residual	5.579E-06	4	1.395E-06	-	-
Total	0.11	17	6.615E-03	-	-
Lack of fit tests					
Linear	6.271E-04	9	6.967E-05	49.95	0.0009
Quadratic	1.295E-04	3	4.317E-05	30.95	0.0032
Cubic	0.000	0	0	-	-
Pure error	5.579E-06	4	1.395E-06	-	-
Source	Root MSE	R^2	Adjusted R^2	Predicted R^2	Press
Model summary statistics					
Linear	6.976E-03	0.9675	0.9600	0.9458	1.055E-03
Quadratic	4.393E-03	0.9931	0.9841	0.8931	2.081E-03
Cubic	1.181E-03	0.9997	0.9989	-	-

Table 4: Sequential model fitting for carboxyl content of the samples prepared

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value (Prob > F)
Sequential model sum of squares					
Mean	0.45	1	0.45		
Linear	0.14	3	0.048	71.17	< 0.0001
Quadratic	8.718E-03	6	1.453E-03	93.00	< 0.0001
Cubic	8.543E-05	3	2.848E-05	4.76	0.0829
Residual	2.393E-05	4	5.983E-06	-	-
Total	0.60	17	0.035	-	-
Lack of fit tests					
Linear	8.804E-03	9	9.782E-04	163.49	< 0.0001
Quadratic	8.543E-05	3	2.848E-05	4.76	0.0829
Cubic	0.000	0	0	-	-
Pure error	2.393E-05	4	5.983E-06	-	-
Source	Root MSE	R^2	Adjusted R^2	Predicted R^2	Press
Model summary statistics					
Linear	0.026	0.9426	0.9294	0.8810	0.018
Quadratic	3.953E-03	0.9993	0.9984	0.9909	1.404E-03
Cubic	2.446E-03	0.9998	0.9994	-	-

Table 5: Analysis of variance results of the quadratic regression model for carbonyl content

Source	Coefficient estimate of coded factors	Coefficient estimate of actual factors	Standard error	*p*-value (Prob > F)
Model*	0.081	-0.15	1.768E-03	<0.0001
X_1*	0.048	0.21	1.397E-03	<0.0001
X_2	2.312E-03	2.290E-03	1.397E-03	0.1801
X_3*	6.437E-03	3.196E-03	1.397E-03	0.0043
X_{12}	-2.500E-05	-4.000E-06	1.976E-05	0.9912
X_{13}	1.475E-03	5.900E-04	1.976E-03	0.5234
X_{23}	-2.450E-03	-9.800E-06	1.976E-03	0.3015
X_1^2	-2.105E-03	-0.034	1.926E-03	0.3583
X_2^2*	-8.330E-03	-1.333E-05	1.926E-03	0.0060
X_3^2**	-5.280E-03	-5.280E-05	1.926E-03	0.0431
Adequacy precision	32.455			
Coefficient of variation, CV	5.94			

Note: X_1: Active chlorine content (g/25 g starch); X_2: Amplitude of ultrasound (%); X_3: Sonication time (min).

* *p* values <0.01 were considered to be significant

** *p* values < 0.05 were considered to be significant

Table 6: Analysis of variance results of the quadratic regression model for carboxyl content

Source	Coefficient estimate of coded factors	Coefficient estimate of actual factors	Standard error	*p*-value (Prob > F)
Model*	0.17	-0.11	1.768E-03	<0.0001
X_1*	0.13	-0.14	1.397E-03	<0.0001
X_2*	0.012	2.778E-03	1.397E-03	<0.0001
X_3*	0.037	2.352E-03	1.397E-03	<0.0001
X_{12}*	9.688E-03	1.550E-03	1.976E-05	0.0017
X_{13}*	0.039	0.015	1.976E-03	<0.0001
X_{23}	-1.875E-03	-7.500E-06	1.976E-03	0.3744
X_1^2*	0.015	0.23	1.926E-03	0.0001
X_2^2*	-0.012	-1.956E-05	1.926E-03	0.0004
X_3^2*	-0.015	-1.451E-04	1.926E-03	0.0001
Adequacy precision	110.532			
Coefficient of variation, CV	2.44			

Note: X_1: Active chlorine content (g/25 g starch); X_2: Amplitude of ultrasound (%); X_3: Sonication time (min).

* *p* values <0.01 were considered to be significant

** *p* values < 0.05 were considered to be significant

adequate signal. The coefficient of variation (CV) values for carbonyl and carboxyl contents of the samples prepared were 5.94 and 2.44 respectively. These low values revealed that the conducted experiments had a very high degree of precision and a good deal of the reliability.

The regression equation for coded values Equation (5) and actual experimental values Equation (6) for carbonyl content (%) which indicates the degree of oxidation of starch as a function of active chlorine content (g/25 g starch), X_1, amplitude of ultrasound (%), X_2 and sonication time (min), X_3 is regressed as follows:

$$Y_{code} = 0.081 + 0.048X_1 + 2.312E\text{-}03X_2 + 6.437E\text{-}03X_3 - 2.105E\text{-}03X_1^2 - 8.330E\text{-}03X_2^2 - 5.280E\text{-}03X_3^2 - 2.500E\text{-}05X_1X_2 + 1.475E\text{-}03X_1X_3 - 2.450E\text{-}03X_2X_3 \quad (5)$$

$$Y_{actual} = -0.15 + 0.21X_1 + 2.290E\text{-}03X_2 + 3.196E\text{-}03X_3 - 0.034X_1^2 - 1.333E\text{-}05X_2^2 - 5.280E\text{-}05X_3^2 - 4.000E\text{-}06X_1X_2 + 5.900E\text{–}04X_1X_3 - 9.800E\text{–}06X_2X_3 \quad (6)$$

And, the regression equation for coded values Equation (7) and actual experimental values Equation (8) for carboxyl content (%) which indicates the degree of oxidation of starch as a function of active chlorine content (g/25 g starch), X_1, amplitude of ultrasound (%), X_2 and sonication time (min), X_3 is regressed as below:

$$Y_{code} = 0.17 + 0.13X_1 + 0.012X_2 + 0.037X_3 + 0.015X_1^2 - 0.012X_2^2 - 0.015X_3^2 + 9.688E\text{–}03\ X_1X_2 + 0.039X_1X_3 - 1.875E\text{–}03X_2X_3 \quad (7)$$

$$Y_{actual} = -0.11 - 0.14X_1 + 2.778E\text{–}03X_2 + 2.352E\text{–}03X_3 + 0.23X_1^2 - 1.956E\text{–}05X_2^2 - 1.451E\text{–}04X_3^2 + 1.550E\text{–}03X_1X_2 + 0.015X_1X_3 - 7.500E\text{–}06X_2X_3 \quad (8)$$

Effect of process variables on the degree of starch oxidation: Determination of carbonyl and carboxyl contents of oxidized starch is important as it indicates the degree or extent of starch oxidation. The main factors which affected the degree of starch oxidation in the present study were active chlorine content, % amplitude of ultrasound and sonication time. Statistically designed experiments with different combinations of these factors were carried out in this section in order to study the combined effect of these factors. In this study, it was found that active chlorine content showed the greatest influence on degree of starch oxidation, followed by sonication time and % amplitude of ultrasound.

Effect of active chlorine content and % amplitude of ultrasound on degree of starch oxidation: The carbonyl and carboxyl contents as a function of active chlorine content (ranged from 0.25 g/25 g starch to 0.75 g/25 g starch) and amplitude of ultrasound (ranged from 50% to 100%) at a constant sonication time of 20 min are shown in the response surface and contour plots in Figure 1(a) and Figure 2(a), respectively. It can be observed that both carbonyl and carboxyl contents generally increased with increasing active chlorine content. However, increased in % amplitude of ultrasound had no effect on carbonyl content, but it had significant influence on carboxyl content ($P<0.01$). According to Santos *et al.* (2009), to achieve the cavitation threshold a minimum intensity is required. This indicates that higher amplitudes are not always necessary to obtain the desired results. Our results suggest that amplitude of ultrasound at 50% is sufficient to promote cavitation to oxidized hydroxyl groups of starch molecules to carbonyl groups and then consecutively to carboxyl groups and the carbonyl groups have been converted promptly to carboxyl groups once they are formed, resulting in no significant changes in carbonyl contents with further increase in % amplitude of ultrasound.

Effect of active chlorine content and sonication time on degree of starch oxidation: Figure 1(b) and Figure 2(b) represent the response surface and contour plots of carbonyl and carboxyl contents of oxidized starches, respectively, as a function of active chlorine content and sonication time. Generally, both carbonyl and carboxyl contents were significantly affected by active chlorine content and sonication time. It can be seen that the carbonyl and carboxyl contents increased with progressive increase in active chlorine. This observation is in line with the study of Wang and Wang (2003), who reported that the degree of starch oxidation increased with oxidant concentration. At higher active chlorine level, larger amount of carbonyl and carboxyl groups were formed as more oxidant was available for oxidation reaction. It can also be noted that carbonyl and carboxyl contents were increased with longer sonication time. However, the effect of sonication time was not as profound as active chlorine level in increasing the carbonyl and carboxyl contents.

Effect of % amplitude of ultrasound and sonication time on degree of starch oxidation: The response surface and contour plots which show the carbonyl and carboxyl contents of oxidized starches as a function of % amplitude of ultrasound and sonication

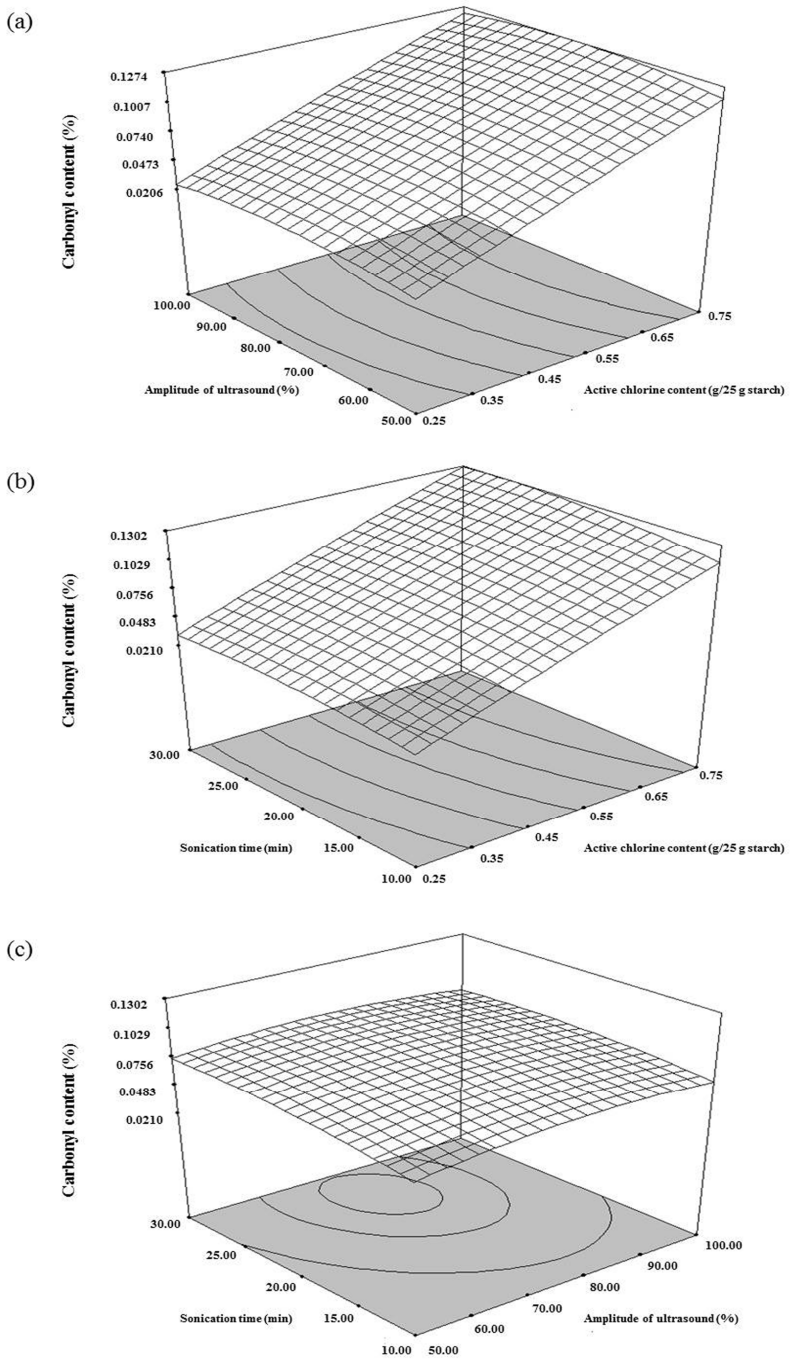

Fig 1: Response surface and contour plots of carbonyl content of samples studied as a function of (a) active chlorine content and amplitude of ultrasound, sonication time fixed at 20 min, (b) active chlorine content and sonication time, amplitude of ultrasound fixed at 75%, (c) amplitude of ultrasound and sonication time, active chlorine content fixed at 0.50 g/25 g starch

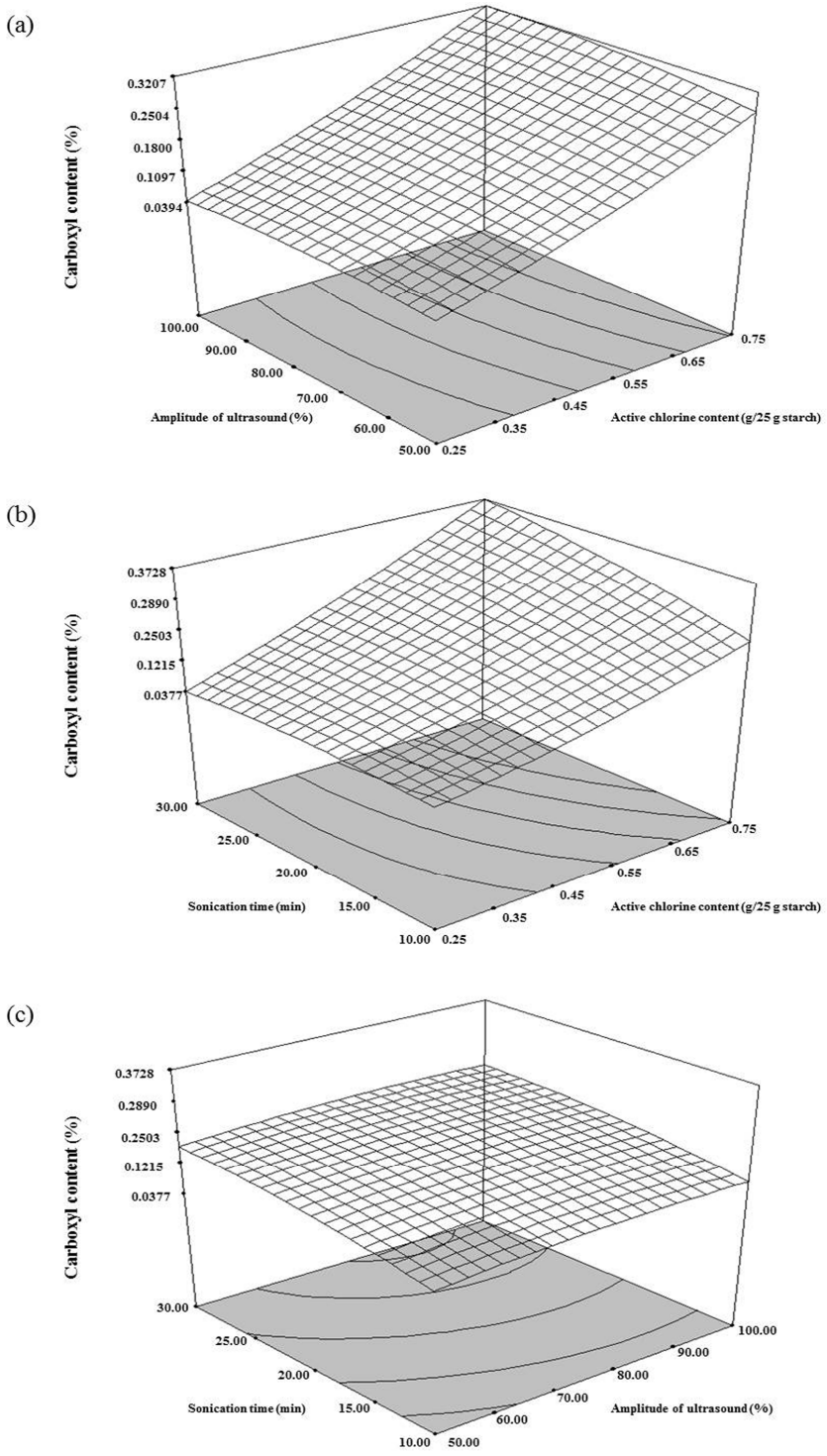

Fig 2: Response surface and contour plots of carboxyl content of samples studied as a function of (a) active chlorine content and amplitude of ultrasound, sonication time fixed at 20 min, (b) active chlorine content and sonication time, amplitude of ultrasound fixed at 75%, (c) amplitude of ultrasound and sonication time, active chlorine content fixed at 0.50 g/25 g starch

Table 7: Comparison of predicted and measured values for carbonyl and carboxyl contents

Description		Starch sample 1	Starch sample 2
Active chlorine content (g/25 g starch)		0.375	0.625
Amplitude of ultrasound (%)		75	75
Sonication time (min)		15	25
Carbonyl content (%)	Predicted value	0.0490	0.1023
	Measured value	0.0501	0.1049
	Absolute average deviation, AAD	2.3371	
	Coefficient of determination, R^2	0.993	
Carboxyl content (%)	Predicted value	0.0903	0.2518
	Measured value	0.1001	0.2667
	Absolute average deviation, AAD	7.6885	
	Coefficient of determination, R^2	0.977	

time are showed in Figure 1(c) and 2(c), respectively. As shown, increase in % amplitude of ultrasound and sonication time resulted in only slight increase in both responses. On the other hand, results show that higher % ultrasound amplitude and longer sonication time may have a negligible effect on carbonyl and carboxyl contents.

Verification of developed regression models: To verify the regression equation developed from the Box-Behnken design, duplicate experiments with two different oxidation conditions were carried out. The levels chosen, predicted values, measured values, absolute average deviation (AAD) values and coefficient of determination (R^2) values for both carbonyl and carboxyl content (%) are tabulated in Table 7. Results show that for both samples tested, the predicted and measured carbonyl contents were 0.0490% and 0.0501% (Sample 1); 0.1023% and 0.1049% (Sample 2) whilst the predicted and measured carboxyl contents were 0.0903% and 0.1001% (Sample 1); 0.2518% and 0.2667% respectively (Sample 2). Obviously, the predicted values were close to the corresponding measured values. It was found that the AAD values between the predicted and measured values were small and the R^2 values were close to 1.0. The AAD values were 2.3371 and 7.6885 whilst the R^2 values were 0.993 and 0.973 for carbonyl and carboxyl contents respectively. These results indicate high accuracy of the model and the regression equations proposed show the true behavior of the process. Hence, it could be concluded that with the regression equation developed, it would be possible to predict the degree of starch oxidation.

Conclusions

The developed models were capable of predicting the degree of starch oxidation and these data might be useful as a reference for the application of oxidized starches in food and non-food industries. Among the three variables studied, ANOVA results showed that active chlorine content showed the most influencing effect on the sonicated hypochlorite-

oxidized starch, followed by sonication time and amplitude of ultrasound.

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